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PCR-Based Diagnosis of the Filipino (−−^{FIL}) and Thai (−−^{THAI}) α-Thalassemia-1 Deletions

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In southeast Asia, the carrier frequency of two-gene α-thalassemia deletions is quite high, ranging from 4% to 14% depending on the population. The most common α-thalassemia-1 deletion is the so-called southeast Asian deletion (−−^{SEA}). In addition, a significant proportion of cases involve two other deletions, the Filipino (−−^{FIL}) and Thai (−−^{THAI}) deletions. In this report, we identify the deletion breakpoints for the (−−^{FIL}) and (−−^{THAI}) deletions, and describe PCR-based protocols for rapid and reliable DNA diagnosis of these deletions. Am. J. Hematol. 63:54–56, 2000. © 2000 Wiley-Liss, Inc.

INTRODUCTION

The most common causes of α-thalassemia are deletions that remove one (−α) or both (−−) of the two functional α-globin genes [1]. Carriers of these deletions have mild microcytosis, with or without anemia, but otherwise are healthy. However, if both partners are carriers, they could be at reproductive risk for Hb Bart's hydrops fetalis syndrome (−−/−−) or Hb H disease (−−/−α). In southeast Asia, the carrier rate for two-gene cis deletions (−−/αα) is high, ranging from 4% to 14% depending on the population [2,3]. The most common deletion of this type is the so-called southeast Asian α-thalassemia-1 deletion (−−^{SEA}). This deletion spans approximately 20.5 kb, removing both functional α-globin genes while leaving the ζ2-globin gene intact [4]. Adult carriers of the (−−^{SEA}) deletion have low but detectable levels of ζ-globin chains in their erythrocytes, the presence of which forms the basis of a rapid and reliable immunofluorescence test [5]. Definitive diagnosis of the (−−^{SEA}) deletion is usually based on Southern hybridization using the ζ-globin gene probe, or PCR using primers that flank the deletion breakpoints [6].

Although the (−−^{SEA}) deletion is by far the most prevalent *cis* deletion in southeast Asia, several other deletions have been reported in the literature. In particular, the Filipino (−−^{FIL}) and Thai (−−^{THAI}) deletions are relatively common among certain southeast Asian populations. These deletions, originally described by Fischel-Ghodsian and colleagues, span approximately 30–38 kb and remove the ζ2-globin gene as well as both α-globin genes [7]. Carriers of these deletions cannot be identified using the anti-ζ immunofluorescence assay or by Southern hybridization with the ζ-globin gene probe. Detection of these deletions requires probes that lie beyond the 5′ and 3′ deletion breakpoints, such as probes L0 and 3′ αHVR [7]. In this report, we identify the deletion breakpoints for the (−−^{FIL}) and (−−^{THAI}) deletions, and describe PCR-based protocols for rapid and reliable DNA diagnosis of these deletions.

MATERIALS AND METHODS

Patient Samples

Carriers of the (−−^{FIL}) and (−−^{THAI}) were identified by Southern hybridization using the L0 probe and the diagnostic restriction endonucleases *Sst*I and *Eco*RI [7]. *Sst*I generates a normal fragment of 5.0 kb and similar-sized abnormal fragments of 7.4 and 8.0 kb for the (−−^{FIL}) and (−−^{THAI}) deletions, respectively. The *Eco*RI hybridization pattern was used to distinguish between the (−−^{FIL}) and (−−^{THAI}) deletions, since only the (−−^{THAI}) deletion

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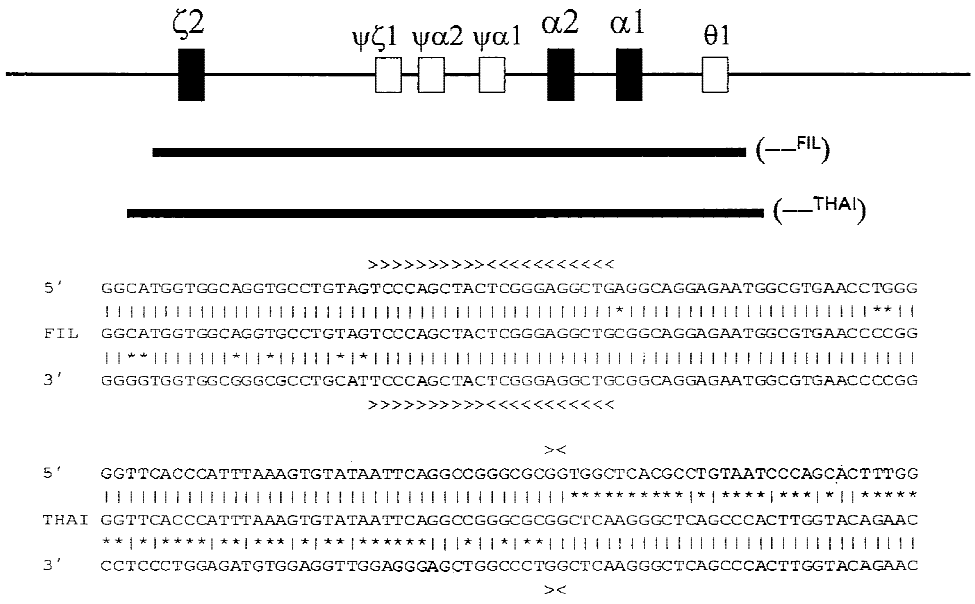

Fig. 1. Map of the ζ-α globin gene cluster showing the relative positions of the (−−FIL) and (−−THAI) deletions and the sequences of the deletion junctions compared to the normal 5′ and 3′ breakpoint sequences. The sequences within which the crossovers occurred are highlighted.

is associated with an abnormal *Eco*RI fragment length (normal = 0.8 kb, abnormal = 8.0 kb).

PCR-Based Diagnosis of the (−−FIL) Deletion

Primers F1 (5′-CTGCCCTTCACACCTCAGACA-3′, GenBank Z84721 positions 11961–11981) and F2 (5′-GCAATCTTGGCTCACTGCAGG-3′, complementary to GenBank Z69706 positions 278–258) were used to amplify a 597 bp fragment that spans the (−−FIL) deletion junction, while primers F2 and F3 (5′-GAAATGGTATTCTCAAGGTGACAC-3′, GenBank Z69706 positions 2–25) amplify a 277 bp fragment that serves as a control for the normal allele. The 100 μL multiplex PCR contains 10 mM Tris·HCl pH 8.3, 50 mM KCl, 2.0 mM $MgCl_2$, 16.5 mM ammonium sulfate, 0.01% gelatin (w/v), 200 μM each dNTP, 10% DMSO, 2.5 U AmpliTaq Gold, 0.22 μM primer F1, 0.73 μM primer F2, 0.45 μM primer F3, and 100–500 ng genomic DNA. The PCR is run in a Perkin-Elmer 2400 or 9600 thermocycler for 35 cycles of 94°C for 40 s, 63°C for 20 s, and 72°C for 2 min. The PCR products are analysed by gel electrophoresis (2.5% agarose or 5% polyacrylamide) and visualized by ethidium bromide staining and UV fluorescence.

PCR-Based Diagnosis of the (−−THAI) Deletion

Primers T1 (5′-TGACTGCATCATAATTCCAGCAG-3′, GenBank Z84721 positions 10504–10523) and T2 (5′-TGAGGCAGGAGATTCGCTTGA-3′, complementary to GenBank Z69706 positions 1478–1458) amplify a 480 bp fragment that is diagnostic of the (−−THAI) deletion, while primers T1 and T3 (5′-GTAGAGATGGTGTTTTGCCATGT-3′, complementary to GenBank Z84721 positions 11140–11118) amplify a 637 bp fragment that serves as a control for the nondeleted allele. PCR conditions were similar to those used to detect the (−−FIL) deletion, with modifications to the $MgCl_2$ concentration (1.5 mM), primer concentrations (T1 = 0.40 μM, T2 = 0.15 μM, T3 = 0.27 μM), primer annealing temperature (61°C), and number of cycles (32).

RESULTS AND DISCUSSION

The known sequence of the ζ-α globin gene cluster was used to design primers that flank the approximate deletion breakpoints originally defined by Fischel-Ghodsian et al. [8]. Primers F1 and F2 were used to amplify a 597 bp fragment that spans the (−−FIL) deletion junction. The nucleotide sequence of the junction fragment was compared to the normal 5′ and 3′ sequences, and the cross-over was determined to be within a region of 21 bp which is identical between the 5′ and 3′ sequences (Fig. 1). The 5′ breakpoint lies approximately 1.3 kb upstream of the initiation codon of the ζ2-globin gene and the 3′ breakpoint lies approximately 5.6 kb downstream of the initiation codon of the α1-globin gene. The total length of the deletion is 30.7 kb.

Both the 5′ and 3′ breakpoints of the (−−FIL) deletion lie within *Alu* repetitive elements that share 88% sequence homology and are oriented in the same direction. Accordingly, the (−−FIL) deletion probably resulted from homologous recombination between two *Alu* sequences. The ζ-α globin gene cluster contains a disproportionately high concentration of *Alu* repeat elements, and *Alu–Alu* recombination events have been suggested as a mechanism for the formation of other deletions within the cluster [1,4].

At the time of this publication, three other groups have independently identified the same deletion breakpoints

and established PCR protocols for detection of the ($--^{FIL}$) deletion [9–11]. It should be noted, however, that one of the groups mistakenly identified the ($--^{FIL}$) deletion as the ($--^{THAI}$) deletion [12,13]. Although the ($--^{FIL}$) deletion is several kilobases smaller than the ($--^{THAI}$) deletion, they can easily be mistaken for each other due to similar hybridization patterns with several restriction endonucleases [7,12].

The actual ($--^{THAI}$) deletion was characterized using primers T1 and T2 to amplify a 480 bp fragment that spans the deletion junction. The sequence of this fragment was then used to deduce the 5′ and 3′ deletion breakpoints (Fig. 1). The 5′ deletion endpoint lies approximately 3.0 kb upstream of the initiation codon of the ζ2-globin gene and the 3′ breakpoint lies approximately 6.6 kb downstream of the initiation codon of the α1-globin gene. The total length of the deletion is 33.4 kb, in good agreement with the original mapping studies [7]. Unlike the ($--^{FIL}$) deletion, the 5′ and 3′ breakpoints of the ($--^{THAI}$) deletion do not share significant sequence homology. The 5′ deletion breakpoint lies within a partial *Alu* repeat element that shares only 32% sequence homology with the 3′ breakpoint sequence.

The development of PCR-based protocols for detecting common α-thalassemia-1 deletions is of significant clinical importance for carrier screening and prenatal diagnosis of pregnancies at risk for Hb Bart's hydrops fetalis [2]. Approximately 5% of Ontario's 11 million residents are of southeast Asian descent (Chinese, Thai, Laotian, Filipino, Vietnamese) and are at high risk for being carriers of α-thalassemia-1 deletions. Over the past decade, we have identified more than seven hundred carriers of α-thalassemia-1 deletions ($--/\alpha\alpha$), 160 individuals with Hb H disease ($--/-\alpha$ or $--/\alpha^{T}\alpha$), and 34 cases of hydrops fetalis due to homozygous α-thalassemia-1 ($--/--$). Among those of southeast Asian descent, the vast majority of cases involve the ($--^{SEA}$) deletion. The second most common α-thalassemia-1 deletion is the ($--^{FIL}$) deletion, which accounts for approximately 15% of the α-thalassemia-1 deletions and is found predominantly among those of Filipino descent. The ($--^{THAI}$) deletion is approximately 7-fold less common than the ($--^{FIL}$) deletion.

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REFERENCES

1. Higgs DR, Vickers MA, Wilkie AOM, Pretorius I-M, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human α-globin gene cluster. Blood 1989;73:1081.
2. Chui DHK, Waye JS. Hydrops fetalis caused by α-thalassemia: an emerging health care problem. Blood 1998;91:2213.
3. Lau Y-L, Chan L-C, Chan Y-YA, Ha S-Y, Yeung C-Y, Waye JS, Chui DHK. Prevalence and genotypes of α- and β-thalassemia carriers in Hong Kong—implications for population screening. N Engl J Med 1997;336:1298.
4. Nicholls RD, Fischel-Ghodsian N, Higgs DR. Recombination at the human α-globin gene cluster: sequence features and topological constraints. Cell 1987;49:369.
5. Tang W, Luo H-Y, Eng B, Waye JS, Chui DHK. Immunocytological test to detect adult carriers of ($--^{SEA}$/) deletional α-thalassemia. Lancet 1993;342:1145.
6. Bowden DK, Vickers MA, Higgs DR. A PCR-based strategy to detect the common severe determinants of α-thalassemia. Br J Haematol 1992;81:104.
7. Fischel-Ghodsian N, Vickers MA, Ship M, Winichagoon P, Higgs DR: Characterization of two deletions that remove the entire human ζ-α globin gene complex ($--^{THAI}$ and $--^{FIL}$). Br J Haematol 1988;70:233.
8. Flint J, Thomas K, Micklem G, Raynham H, Clark K, Doggett NA, King A, Higgs DR. The relationship between chromosome structure and function at a human telomere region. Nat Genet 1995;15:252.
9. Lee L, Hsia YE, Donlon TA, Hunt JA. Determination of the breakpoints of the common α-thalassemia deletion in Filipinos in Hawaii. Br J Haematol 1999;104:284.
10. Ko T-M, Tseng L-H, Kao C-H, Lin Y-W, Hwa H-L, Hsu P-M, Li S-F, Chuang S-M. Molecular characterization and PCR diagnosis of Thailand deletion of α-globin gene cluster. Am J Hematol 1998;57:124.
11. Hattori Y, Okayama N, Ohba Y, Yamashiro Y, Yamamoto Ku, Tsukimoto I, Kohakura M. The precise breakpoints of a Filipino-type α-thalassemia-1 deletion found in two Japanese. Hemoglobin 1999;23:239.
12. Higgs DR, Ayyub H, Chong SS. The $--^{THAI}$ and $--^{FIL}$ determinants of α-thalassemia in Taiwan. Am J Hematol 1999;60:80.
13. Ko T-S, Li S-F. Molecular characterization of the $--^{FIL}$ determinant of alpha-thalassemia. Am J Hematol 1999;60:173.